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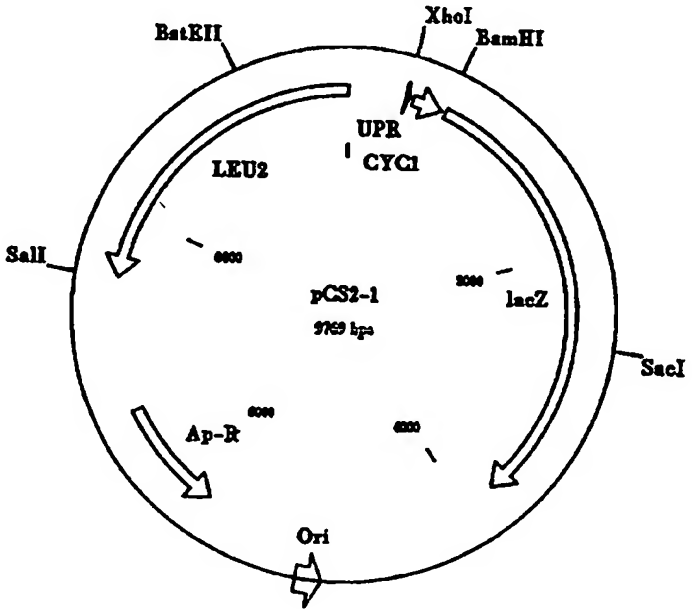

(57) Abstract

The current invention concerns a new method for the detection of malfolded proteins; a method for the measurement of the amount of malfolded protein produced; and a method for the detection of compounds that influence the malfolding.

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Method for detection of malfolded protein

The current invention concerns a new method for the detection of malfolded proteins; a method for the measurement of the amount of malfolded protein produced; and a method for the detection of compounds that influence the malfolding.

When malfolded proteins accumulate in a cell, the transcription of genes encoding certain proteins is induced in the nucleus. This 'unfolded-protein response' was first studied in animal cells, where the expression of BiP/GRP78, GRP94, PDI/ERp59 and ERp72 is induced by a variety of different treatments that cause an increase in malfolded protein. The response to the accumulation of malfolded proteins is regulated at the transcriptional level with the aid of certain enhancer elements.

A similar response to the accumulation of malfolded protein has also been observed in *Saccharomyces cerevisiae*, where the expression of a similar set of genes is induced, including Bip (encoded by KAR2), PDI, Eug1p, and peptidyl-prolyl cis-trans isomerase. Using deletion analysis with the KAR2 promoter, a 22 bp element, the unfold-protein-response element (UPR) has been identified. This UPR is necessary and sufficient to activate transcription in response to the accumulation of malfolded protein (Shamu *et al.*, Trends in Cell Biol. (1994), 4, 56-60).

The biological function of a protein is governed by specifically adopted three-dimensional structures. Malfolded or aggregated proteins fail to acquire the necessary conformation required for the activity of a protein. Usually, the accumulation of malfolded proteins in the cell is connected directly or indirectly to several diseases like Alzheimer's disease (AD) and human prion associated diseases like Creutzfeldt-Jacob disease and Gertsmann-Sträussler-Scheinker disease. Another example for the effect of a malfolded protein is the tumor supressor protein p53 that is a negative regulator of cell growth (Donehower & Bradley, Biochim. Biophys. Acta (1993), **1155**, 81-205). The majority of human tumors express mutant p53 proteins. Hence, it is believed, that mutant p53 proteins are malfolded, or at least partially denatured.

Alzheimer's Diseases, for example, is pathologically connected to the appearance of cerebrovasular amyloid deposits. The major protein found in these deposits is a 39-42 amino acid peptide named β-amyloid peptide mainly in the form of cross β-conformation. From cDNA analysis it became evident that the β-amyloid peptide is derived from a amyloid

precursor protein (APP) through *in vivo* proteolysis. APP is produced in numerous cell types and normally does not aggregate. In contrast thereto synthetic molecules of β-amyloid have been shown to aggregate (Barrow *et al.*, J. Mol. Biol. (1992), 225, 1075-1093). It is thought that, in the cell, proteins which initially malfold ultimatively undergo aggregation but protein that aggregate are not necessarily malfolded.

With this knowledge at hand, it would be highly appreciated to have a test that allows a fast and easy way to screen compounds for their influence on malfolding of proteins, for example for their ability to minimize the production of a certain malfolded protein, to reduce the negative effects of the malfolded protein produced or to counterbalance effects that cause a protein to become malfolded.

Summary of the invention

Surprisingly, it has now been found that the influence of compounds on the malfolding of proteins can be monitored *via* an easily measurable reporter gene product if the DNA coding for the malfolded protein is connected operable to a signal sequence that induces the transport of this protein through the endoplasmic reticulum (ER) and if one or more UPR elements are connected operable to the reporter element. The inventive method also provides an easy method for determining whether unusual aggregation of a protein is related to malfolding or not. For example in the case of the β-amyloid peptide it has been surprisingly found, that aggregation that occurs *in vivo* and *in vitro* is associated to malfolding and can be monitored *in vivo* using the inventive system. Furthermore, the rate of aggregation of different β-amyloid peptides (shortened, inverted or from different organisms) as estimated *in vitro* is closely correlated to the rate of malfolding measured with the inventive system. Therefore, it is also possible to monitor the influence of mutations on the rate of malfolding and the effect associated therewith.

Detailed description of the invention

The present invention concerns a host transformed with at least a first and a second expression cassette, wherein

- the first expression cassette comprises one or more unfolded-protein-response elements (UPR) operably linked to a reporter element;

- the second expression cassette comprises a promoter operably linked to a signal sequence, to a DNA encoding a protein whose malfolding is to be monitored and to a terminator; and

wherein these first and second expression cassettes are not naturally occurring in the host and wherein the protein to be monitored in the second expression cassette is selected from the group consisting of a prion, p53, β-amyloid peptide and functional derivatives thereof.

Hosts

A suitable host, according to the present invention, may be any host which is capable of an 'unfolded-protein response', like hosts that secrete proteins *via* the ER. Examples for suitable hosts are plant, insect, mammalian, fungal, or animal cells. Preferred are fungal cells, more preferred is a yeast cell and most preferred is *Saccharomyces cerevisiae*.

Suitable yeast strains according to the invention include strains of *S. cerevisiae* containing the endogenous two-micron plasmid or such strains which have been cured of said endogenous two-micron plasmid (see EP-A-340170).

The host can be transformed with the required first and second expression cassette by means commonly used in genetic engineering and as described, for example, in *Sambrook et al.*, Molecular Cloning: A laboratory manual, 2nd Edn. 1989.

Expression cassettes

An unfolded-protein-response elements (UPR), as used in the first expression cassette, is a fragment of DNA that is involved in the transcription initiation in case malfolded protein accumulates. UPR elements can be isolated, for example, from the initiation region of a protein that is involved originally in the response to the accumulation of malfolded protein as for example from BiP, FKB2, BiP/GRP78, GRP94, PDI/ERp59 and ERp72 (Shamu *et al.*, Trends in Cell Biol. (1994), 4, 56-60).

A preferred UPR is isolated from BiP and more preferably comprises a DNA sequence as depicted in SEQ ID NO 1 or is a functional equivalent thereof. Functional equivalent has the meaning of a DNA sequence that is derived from SEQ ID NO 1 by replacement of some of the restriction sites at one or both ends or by replacement of nucleotides, e.g. 1 to 5, that do not interfere with the activity to respond to the accumulation of unfolded protein. For a

more strong response to the accumulation the UPR may be present in more than one copy, for example in 2 to 5 copies.

In a preferred first expression cassette of the invention, the reporter element comprises a promoter operably linked to a DNA that is transcribed under the control of this promoter, and to a terminator.

The promoter in this reporter element can be of almost any origin. It is for example possible to use a tightly regulated promoter or the promoter that is naturally adjacent to said DNA, like the β-galactosidase or luciferase promoter, or the CYC1, the Gal1/GAL19, PHO5 or the KAR2 promoter. In a preferred embodiment of the invention the promoter is selected from the group consisting of the CYC1 and the KAR2 promoter.

A suitable DNA that is transcribed under the control of this promoter usually causes an effect that can be measured easily during or after transcription or translation. Preferred are transcription or translation products that can be measured easily e.g. *via* the measurement of the amount of protein, mRNA or DNA produced; or that cause an effect that can be measured easily, e.g. cell growth, an enzymatic reaction or color. Hence, the transcribed DNA codes, for example, for a protein that is produced in an amount that is related to the amount of activation of said promoter and that is, e.g., not produced elsewhere in the chosen host under the applied conditions. Examples for suitable proteins are the metallothionein that is encoded by the yeast CUP1 gene, β-galactosidase or luciferase. Preferred is luciferase and β-galactosidase.

A suitable terminator for this first expression cassette usually contains also the proper signals for transcription termination. This terminator may be naturally linked to the transcribed DNA or may be introduced from a different origin like the PHO5, the α-factor or the SUC2 terminator.

This first expression cassette may, for example, contain additionally a DNA sequence encoding a signal peptide as defined below.

The promoter for the second expression cassette according to the invention can be almost any promoter homologous or heterologous to the host. It is, for example, possible to use the promoter originally linked to the DNA encoding the protein whose malfolding is to be monitored; or it can be promoter commonly used in genetic manipulations of the host. The

promoter can be inducible or not inducible like the CUP1, GAPDH, GAPFL, GAL(1/10), PYK, TPI, ADH, PRC1 and PGK promoter. Preferred is the GAPFL promoter or a functional derivative thereof.

If a inducible promoter is used, the host can be grown under optimal growth conditions and the expression of the protein whose malfolding is to be monitored can be induced at a desired moment.

The signal sequence usually is chosen in accordance with the host that is used in the test. A suitable signal sequence can be derived from any gene coding for a polypeptide that is ordinarily secreted. Examples are the SUC2, CPY, α-factor, KEX1, PHO5 and the glucoamylase signal sequence.

The protein whose malfolding is to be monitored are prions, p53, the β-amyloid peptide or functional derivatives thereof. In a preferred embodiment of the invention the protein to be monitored comprises the β-amyloid peptide or a functional derivative thereof. The expression functional derivative denotes a peptide that is derived from the original malfolded peptide by minor changes, for example through up to 10 amino acid replacements, addition, or deletion, but wherein the properties in respect to malfolding are comparable or equal.

The terminator used in the second expression cassette can be the terminator naturally adjacent to the DNA coding for the protein whose malfolding is to be monitored, or another terminator as described above.

The promoter, the DNA sequence coding for the signal peptide, the DNA sequence coding for the polypeptide and the DNA sequence containing transcription termination signals are operably linked to each other, i.e. they are juxtaposed in such a manner that their normal functions are maintained. The array is such that the promoter effects proper expression of the signal sequence-polypeptide gene complex, the transcription termination signals effect proper termination of transcription and polyadenylation and the signal sequence is linked in the proper reading frame to the polypeptide gene in such a manner that the last codon of the signal sequence is directly linked to the first codon of the gene for the polypeptide. The promoter is preferably joined to the signal sequence between the major mRNA start and the ATG naturally linked to the promoter gene. The signal sequence has its own ATG for translation initiation. The junction of these sequences may, for example, be effected by

means of synthetic oligodeoxynucleotide linkers carrying the recognition sequence of an endonuclease.

The expression cassettes according to the invention may be inserted into the genome of the host or in form of a stable plasmid like the two micron plasmid. If one or both of the expression cassettes are inserted in form of a stable plasmid, apart from the polypeptide expression cassettes the expression plasmids can comprise a DNA segment originating from two-micron DNA containing the origin of replication or, if a two-micron DNA free strain of yeast is used, total two-micron DNA. The latter type of plasmids is preferred in this case. For example, the plasmids according to the invention contain the complete two-micron DNA in an uninterrupted form, i.e. two-micron DNA is cleaved once with a restriction endonuclease, the linearized DNA is linked with the other components of the vector prior to recircularization. The restriction site is chosen such that normal function of the REP1, REP2 and FLP genes and of the ORI, STB, IR1 and IR2 sites of two-micron DNA as well as small "FLP recognition target" (FRT) sites, located near the center of each inverted repeat (IR) at which the FLP recombinase acts, is maintained. Optionally, the restriction site is chosen such that the D gene of two-micron DNA is kept intact too. Suitable restriction sites are, for example, the unique PstI site located within the D gene and the unique HpaI and SnaBI sites located outside of all of said genes and sites. However, it is likewise possible to insert the expression cassette and further components (cf. below) at different (such as two) restriction sites, especially those mentioned above, within two-micron DNA.

Such a plasmid derivative may comprise only two invertedly repeated FRT sites or an additional, third FRT site. The former kind of plasmid is hereinafter called a "symmetric two micron-like hybrid vector". The latter kind of plasmid is hereinafter called "symmetric two micron-like disintegration vector" despite it is not a real symmetric plasmid but gives rise to a symmetric two micron-like hybrid vector in the yeast cell transformed therewith (EP-A-501 914).

A symmetric two micron-like hybrid vector of the invention does preferentially not contain bacterial or viral DNA sequences, i.e. DNA derived from a bacterial genome, plasmid or virus. However, a two micron-like disintegration vector of the invention may comprise DNA sequences of prokaryotic origin between the two directly repeated FRT sites which are excised from the vector in the transformed yeast cell in which the symmetric two micron-like hybrid vector is generated from the disintegration vector. These DNA sequences are bacterial sequences as described below and can provide to the vector essential structural or

functional features or can also only have the function of filling up the two regions between the two invertedly repeated FRT sites of an unsymmetric two micron-like plasmid derivative or of an "unsymmetric" disintegration vector in order to construct a symmetric two micron-like hybrid vector or a symmetric disintegration vector.

In a preferred embodiment, the two regions between invertedly repeated FRT sites of the circular form of the two-micron DNA have approximately the same length.

Preferably, the expression plasmids according to the invention include one or more, especially one or two, selective genetic markers for the host used in the test and such a marker and (except for symmetric two-micron like hybrid vectors) an origin of replication for a bacterial host, especially *Escherichia coli*.

As to the selective gene markers, any marker gene can be used which facilitates the selection for transformants due to the phenotypic expression of the marker gene. Suitable markers are, for example, those expressing antibiotic resistance or, in the case of auxotrophic host mutants, genes which complement host lesions. Corresponding genes confer, for example, resistance to the antibiotics G418, hygromycin or bleomycin or provide for prototrophy in an auxotrophic yeast mutant, for example the URA3, LEU2, LYS2, HIS3 or TRP1 gene.

As the amplification of the expression plasmids is conveniently done in a prokaryote, such as *E. coli*, a prokaryote, e.g. *E. coli*, genetic marker and a prokaryote, e.g. *E. coli*, replication origin are included advantageously. These can be obtained from corresponding prokaryotic plasmids, for example *E. coli* plasmids, such as pBR322 or a pUC plasmid, for example pUC18 or pUC19, which contain both prokaryotic, e.g. *E. coli*, replication origin and genetic marker conferring resistance to antibiotics, such as ampicillin.

Apart from the polypeptide expression cassette, replication origin(s) and genetic marker(s) the expression plasmids according to the invention contain optionally additional expression cassettes, such as 1 to 3 additional polypeptide expression

The expression plasmids according to the invention are prepared by methods known in the art, for example by linking the polypeptide expression cassette, the DNA fragments containing selective genetic markers for host used in the test and optionally for a bacterial host, the origin(s) of replication for yeast and optionally for a bacterial host, and optionally

additional functional fragments or expression cassettes in the predetermined order using conventional chemical or biological *in vitro* synthesis procedures. Preferentially the plasmids are constructed and prepared using recombinant DNA techniques. For the preparation by recombinant DNA techniques suitable DNA fragments are ligated in vitro in conventional manner. The ligation mixture is then transformed into a suitable prokaryotic or eukaryotic host depending on the nature of the regulatory elements used, and a transformant containing the desired vector is selected according to conventional procedures. The plasmids can be multiplicated by means of the transformed hosts and can be isolated in conventional manner. The choice of the host depends on the regulatory sequences located on the vector. As the expression vectors of the invention preferentially comprise regulatory sequences functional in prokaryotes, e.g. *E. coli*, a prokaryotic host, e.g. *E. coli*, is preferred for the construction and multiplication of the vector.

A further embodiment of the invention concerns an expression cassette comprising a promoter operably linked to a signal sequence, a DNA encoding the β-amyloid peptide or a functional derivative thereof, and to a terminator, a hybrid plasmid comprising said expression cassette. In a preferred form this plasmid is based on the two-micron plasmid of *S. cerevisiae*.

Methods for the construction of these expression cassettes from the different functional fragments are well known in the art and are reviewed, for example, in *Sambrook et al.*, Molecular Cloning: A laboratory manual, 2nd Edn. 1989.

The transformed strains are cultured using methods known in the art.

Corresponding complex culture media which can be used for culturing yeast are known in the art. For examples, such culture media contain tryptone, peptone, meat extracts, malt extracts, yeast extracts, casamino acids, corn steep liquor, soy bean flour, whey, whey hydrolysate etc., and especially mixtures thereof and are optionally additionally supplemented with sugars (e.g. dextrose, glucose, sucrose, galactose etc.), vitamins (e.g. biotin), individual amino acids, inorganic salts (for example, sulfates, chlorides, phosphates and carbonates of sodium, potassium, magnesium and calcium, furthermore corresponding salts of trace elements, such as iron, zinc and manganese) and the like taking into account that all essential components as outlined above are to be present in the medium. A preferred culture medium is the commercially available medium YPD (yeast extract, peptone,

dextrose; cf. Methods Enzymol. **194**, 13) optionally supplemented with inorganic salts and vitamins.

Method for testing

Another embodiment of the invention concerns a method for the determination of the influence of a compound on the appearance of malfolded protein, comprising culturing a transformed host as defined above under suitable conditions, applying the compound to be tested and measuring the amount of reporter gene activation.

This influence may be based, for example, on the inhibition of conditions that induce malfolding of said protein, promotion of the correct folding of said protein or prevention of the aggregation of said protein.

The test can be carried out, for example, in the following form:

- growing the transformed host carrying the two expression cassettes under suitable conditions;
- optionally inducing the expression of the protein whose malfolding is to be monitored, this induction is necessary, for example, if the expression of this protein is under the control of a inducible promoter;
- optionally adding a compound that promotes the malfolding or aggregation, this is necessary, for example, if malfolding of the protein depends on additional compounds,
- adding the test compound; and
- monitoring the transcription or translation of the reporter element, or the transcription or translation products or the effects caused thereby .

In a preferred embodiment of the invention the method is used for the identification of compounds that inhibit the aggregation of β-amyloid peptide. These compounds can act, for example, by

- binding to or modifying the protein and inhibiting thereby the aggregation;
- by binding to or modifying the protein and inhibiting thereby the binding of another compound that initiates or promotes aggregation of the protein; or
- by binding or modifying the compound that initiates or promotes the aggregation of the protein.

Compound

Also enclosed are the new compounds identified using the inventive method and the use of these compounds in a method of treatment and especially for the inhibition of protein aggregation as defined above. These new compounds may be used, for example, in the treatment of cancer or Alzheimer's disease.

Brief description of the drawings

In the following experimental part various embodiments of the present invention are described with reference to the accompanying drawings in which:

Fig. 1 is a schematic illustration of plasmid pCS2-1.
Fig. 2 is a schematic illustration of plasmid pFL38/ CPY.

Examples

The following examples illustrate the invention and should not be construed as a limitation thereof.

All enzymatic reaction like cleavage with restriction enzymes, ligations, transformation, annealing and β-galactosidase assay are standard methods in genetic engineering and are carried out essentially as described in *Sambrook et al.*, Molecular Cloning: A laboratory manual, 2nd Edn. 1989.
The enzyme employed for all PCR reactions is Vent polymerase (New England Bio-Labs).
The Primers are synthesized on a DNA synthesizer.

Example 1: Construction of pPFY7 vector for integration of the lacz expression cassettes, under the control of the unfolded-protein-response element(s) linked to a core promoter, into the yeast chromosome

The plasmid pPFY7 is a pBluescript (Stratagene®) based vector. It encodes the *Saccharomyces cerevisiae* LEU2 gene as a yeast selection marker. It also contains the CYC1p-lacz fusion gene. CYC1p represents the core promoter of the yeast iso-1-cytochrome c (CYC1) gene and lacz encodes the *E. coli* β-galactosidase enzyme.

The plasmid pPFY7 has been obtained as follows.

The plasmid pBluescript SK+ (Stratagene®) is digested with SspI and NaeI and a 2832 bp fragment is isolated by agarose/TBE gel electrophoresis. The fragment is isolated and purified by GeneClean® (Bio 101, CA, USA).

The ~2190 bp *Saccharomyces cerevisiae* LEU2 gene is isolated as a SspI-TthI fragment from the plasmid pRS425 (ACTT 77106). After digesting pRS425 with TthI, the sticky end is flushed with the large fragment of Klenow DNA polymerase which is followed by a partial digest with SspI. The above fragment is isolated and purified by GeneClean ® as above.

The 2832 bp SspI-NaeI fragment and a ~2190 bp SspI-flushed end fragment are ligated and transformed in *E. coli* HB101. DNA obtained from individual transformants are analyzed by restriction enzyme analysis. One clone with the correct restriction fragments is named pLEU2.

The plasmid pLEU2 is completely digested with XhoI and PvuII. A ~4716 bp fragment is isolated as described above. An XhoI-PvuII ~3500 bp fragment is isolated from the plasmid pLG669-Z (Guarente & Ptashne, Proc. Natl. Acad. Sci. USA (1981), 2199-2203) by first digesting with XhoI and then performing partial digestion with PvuII. The two fragments are ligated and transformed in HB101. One clone with the correct restriction fragments is named pPFY7.

Example 2: Construction of plasmids containing lacz expression cassettes, under the control of the unfolded-protein-response element(s) linked to a core promoter

The 22 bp unfolded-protein-response (UPR) element (Mori *et al.*, EMBO J (1992), 11, 2583-2593) is chemically synthesized and consists of double-stranded deoxyoligoribonucleotide linkers (SEQ ID NO. 1). The fragment contains XhoI sticky ends, one of which is maintained as a XhoI site after subcloning in the XhoI digested vector pPFY7. Ligation is performed after linker-tailing.

SEQ ID NO 1:

```
      XhoI
5'    TCGA G GGA ACT GGA CAG CGT GTC GAAA          3'
3'        C CCT TGA CCT GTC GCA CAG CTTT AGCT      5'
                                      XhoI
```

Linker-tailing involves the following steps.

~100 ng of double-stranded deoxyoligoribonucleotides (see SEQ ID NO 1) is ligated to 1 µg of XhoI digested pPFY7 at 4°C for 15 h in the presence of T4 DNA ligase (1µl; 1U/µl; Gibco-BRL, Basel, Switzerland). The ligation mixture is fractionated by electrophoresis on a 1% agarose-Tris-acetate gel. The linear fragment, which contains the ligated linkers, is isolated and purified by GeneClean® (Bio 101, CA, USA). The DNA is re-annealed by incubation at 95°C followed by slow cooling to room temperature and is then transformed in the *E. coli* strain HB101. The DNA obtained from the transformants is analyzed on a 2% agarose gel. The clones which show a distinct increase after digestion with XhoI and BamHI are the ones which have the UPR element in the correct orientation upstream of the CYC1 core promoter. One such plasmid is named pCS2-1 (Figure 1). The insert is confirmed by DNA sequencing using the Applied Biosystems DNA sequencer 370A.

The 22 bp unfolded-protein-response element (see SEQ ID NO 1) is ligated to pCS2-1, again via linker-tailing (as described above). This yields the plasmid pCS2-2 which encodes two copies of the UPR element and is confirmed by DNA sequencing (see above).

The plasmids pCS2-3, pCS2-4 and pCS2-5 are obtained similarly. These encode three, four and five UPR elements, respectively. All inserts are confirmed by DNA sequencing (see above).

Example 3: Chromosomal integration of UPR-CYC1p-lacz fusion genes in the yeast strain W3116

The Bio-Rad gene pulser® is employed for transformation of yeast cells by electroporation (Becker & Guarente, Methods-Enzymol, (1991), **194**, 182-187). The yeast strain W3116 (Mata leu2-3 leu2-112 his3 ura3-52 pcr1-Δ:: HIS3 pep4-Δ1137) (J. Winther, Carlsberg Laboratory, Denmark) is used for all integrations of UPR-CYC1p-lacz gene fusions into the yeast chromosome. W3116 is constructed from the strain W3094 as described in van den Hazel *et al.*, Eur. J. Biochem. (1992), **207**, 277-283) by gene replacement (Winston *et al.*, Methods Enzymol. (1983), **101**, 211-228) using the plasmid pJW1137. This plasmid carries a PEP4 gene (Ammerer *et al.*, Mol. Cell. Biol. (1986), **6**, 2490-2499) with a deletion from the EcoRI to the ClaI restriction site, which takes out the promoter and first 75% of the open-reading-frame.

The UPR-CYC1p-lacz constructs (pCS2-1 to 5) are integrated into the LEU2 locus of BstEII cleaved strain W3116 after linearization of the plasmids with BstEII. Correct gene

integration and gene replacement events are verified by PCR. Subsequently, the amplified fragments are analyzed by agarose gel electrophoresis.

TABLE 1. Yeast strains harboring UPR-CYC1p-lacz fusion on the chromosome

Strain	Genotype	UPR-CYC1p-lacz Fusion
W3116	Mata leu2-3 leu2-112 his3 ura3-52 pcr1-Δ:: HIS3 pep4-Δ 1137	-
W3116-1 × lacz	Mata LEU2::pCS2-1 his3 ura3-52 pcr1-Δ:: HIS3 pep4-Δ 1137	1 × UPR-CYC1p-lacz
W3116-2 × lacz	Mata LEU2::pCS2-2 his3 ura3-52 pcr1-Δ:: HIS3 pep4-Δ 1137	2 × UPR-CYC1p-lacz
W3116-3 × lacz	Mata LEU2::pCS2-3 his3 ura3-52 pcr1-Δ:: HIS3 pep4-Δ 1137	3 × UPR-CYC1p-lacz
W3116-4 × lacz	Mata LEU2::pCS2-4 his3 ura3-52 pcr1-Δ:: HIS3 pep4-Δ 1137	4 × UPR-CYC1p-lacz
W3116-5 × lacz	Mata LEU2::pCS2-5 his3 ura3-52 pcr1-Δ:: HIS3 pep4-Δ 1137	5 × UPR-CYC1p-lacz

Example 4: Protein malfolding in the endoplasmic reticulum, caused by the glycosylation inhibitor tunicamycin, induces UPR-CYC1p-lacz genes

All yeast strains in Table 1 are grown as a pre-culture in SD medium (0.67% yeast nitrogen base without amino acids, 2% glucose) for 24 h at 30°C. An aliquot of the pre-culture (1%) is inoculated in YPD medium (1% bacto-yeast extract, 2% bacto-peptone, 2% glucose) and the cells are grown for 16 h at 30°C (control). For induction of protein malfolding, an aliquot of mid-logarithmic phase cultures are treated with tunicamycin (10µg/ ml; Sigma) for 6 h at 30°C. The cells are harvested and washed with 0.9% NaCl. β-galactosidase activity, measured as o-nitrophenyl-β-D-galacto-pyranoside hydrolysis at 420 nm, is normalized to cell culture density and expressed as arbitrary units. The activity in the control strains (which are not treated with tunicamycin) and harboring the plasmid pPFY7, is set to the value 1. The results, which are an average of three individual integrants (each assay having been performed in duplicate) are depicted in Table 2.

TABLE2. Induction of β–galactosidase by tunicamycin in yeast strains harboring UPR-lacz

Strain	Fusion	β-gal activity (-) tunicamycin	β-gal activity (+) tunicamycin
W3116	-	1	1
W3116-1 × lacz	1 × UPR-CYC1p-lacz	1	1.5
W3116-2 × lacz	2 × UPR-CYC1p-lacz	1	3
W3116-3 × lacz	3 × UPR-CYC1p-lacz	1	12
W3116-4 × lacz	4 × UPR-CYC1p-lacz	1	16
W3116-5 × lacz	5 × UPR-CYC1p-lacz	1	17

Example 5: Construction of a 2-micron plasmid which encodes an expression cassette for wild type CPY

The plasmid pLV9 contains the complete PRC1 gene which encodes the yeast carboxypeptidase Y enzyme (CPY) is constructed as described in Valls *et al.*, Cell (1987), 48, 887-897. The ClaI-HindIII PRC1 fragment (containing the promoter, the coding sequence for preproCPY and the transcription terminator) from pLV9 is isolated and converted to a SalI-SacI fragment (5' to 3') for convenient cloning in the 2-micron-based vector pDP34 (DSM 4473) and the centromere vector pFL38 (ATCC 77203). The manipulations of restriction sites at the 5' and 3' ends of PRC1 are made using SalI and SacI linkers available from Boehringer.

The plasmid pDP34 is an *E. coli-S. cerevisiae* shuttle vector, which contains the complete *S. cerevisiae* 2-micron plasmid and encodes the *S. cerevisiae* URA3 and dLEU2 genes as yeast selection markers (EP-A-340 170).

The plasmid pFL38 is also an *E. coli-S. cerevisiae shuttle vector*, which contains the centromere CEN6, an autonomously replicating sequence from *S. cerevisiae* (ARS) and encodes the *S. cerevisiae* URA3 as a yeast selection marker.

After subcloning of the SalI-SacI PRC1 gene fragment in pDP34 (completely digested with SalI and SacI), one correct plasmid is named pDC13.

The SalI-SacI PRC1 gene fragment is also subcloned in pFL38, which is completely digested with SalI and SacI, to obtain pFL38/ CPY (Figure 2).

Example 6: Construction of mutant CPY precursor genes

The mutations incorporated in the C-terminus of the CPY polypeptide sequence are described in Table 3. The site where precursor pro-CPY is processed by proteinase B to form mature CPY is between the residues Asn111 and Lys112 of the prepro-CPY sequence (shown in the one-letter code N and K in Table 3). The residues which are replaced in the wild type (wt) sequence are underlined.

Table 3. The amino acid replacements in the mutated pro-CPY proteins by PCR-mediated site-directed mutagenesis

CPY variant	Amino acid sequence at the C-terminus of the pro-sequence of CPY	SEQ ID NO of the primers used for mutagenesis
wt (as in pFL38/CPY)	A I E N Y Q L R V N K	-
Mut1	A I E N Y Q K R V N K	2 and 3
Mut2	A I E N L D K R V N K	4 and 3
Mut3	A I E N Y K R D P G K	5 and 3

The mutations are generated by performing PCR-mediated site-directed mutagenesis on a 157 bp MunI-BamHI fragment of PRC1 as comprised in pFL38/CPY (Valls *et al.*, Cell (1987), 48, 887-897). The primers used for the PCRs are depicted in Table 3.

A SalI-MunI fragment from pFL38/CPY (see Figure 2 and Example 5) and the mutated MunI-BamHI fragments (see above) are initially subcloned in pUC19 which is completely digested with SalI and BamHI. The resultant plasmids contain a 1159 bp SalI-BamHI fragment belonging to the 5' end of PRC1. The mutations are confirmed by DNA sequencing (see Example 2).

Example 7: Construction of 2-micron plasmids which encode expression cassettes for mutant CPY precursor genes

The complete mutant genes, encoding the promoter, the coding sequence and the transcription terminator) are assembled as SalI-SacI fragments.

A SalI-BamHI fragment (containing a mutated pro sequence of CPY; as subcloned in pUC19; see Example 7), a BamHI-NcoI (931 bp) and a NcoI-SacI (546 bp) (the latter two fragments are obtained from pFL38/CPY; see Figure 1 and Example 5) are subcloned in pDP34 completely digested with SalI and BamHI. The resulting plasmids are named pDC8 (encoding Mut1 PRC1) , pDC9 (encoding Mut2 PRC1) and pDC10 (encoding Mut3 PRC1). They are used for expression in yeast.

Example 8: The mutant PRC1 genes express malfolded proteins

The plasmids pDC8 (encoding Mut1 PRC1), pDC9 (encoding Mut2 PRC1), pDC10 (encoding Mut3 PRC1) and pDC13 (encoding wt PRC1) are transformed in the strain W3116-3 × lacz via electroporation (see Example 3). The β-galactosidase expression induced by the mutant PRC1 genes is compared to the strain which harbors the wild type-gene (wt-gene). Three individual transformants from each of the above four transformations are grown as in Example 4. Each assay is performed in duplicate. The results are depicted in Table 4. The β-galactosidase activity in the strain YDC13, expressing wt PRC1, is set to the value 1.

TABLE4. Induction of β-galactosidase by mutated pro-CPY molecules in a strain which harbors 3 × UPR-lacz

Plasmid	PRC1 allele	Yeast Transformant	β-gal activity
pDC13	wt	YDC13	1
pDC8	Mut1	YDC8	3
pDC9	Mut2	YDC9	4
pDC10	Mut3	YDC10	2.5

Similar results have been obtained with the strain W3116-4 × lacz.

Example 9: The mutated pro-CPY proteins can also be monitored as malfolded when the lacz reporter is under the control of the complete KAR2 promoter

In order to make the KAR2p-lacz construct, an XhoI-BamHI fragment containing the CYC1 promoter is removed from the plasmid pCS2-1 and is replaced by a 630 bp SalI-BamHI KAR2 promoter fragment (Rose *et al.*, Cell (1989), **57**, 1223-1236). The KAR2 promoter is isolated by PCR using the two primers with SEQ ID NO 6 and 7. The template for PCR is the yeast strain S288C (ATCC 26108). The resulting plasmid is named pCSFOL4. This is integrated in W3116 as described in Example 3. Correct integrations are confirmed (see Example 3). One of these strains is referred to as W3116 KAR2p-lacz.

The plasmids pDC8 (encoding Mut1 PRC1), pDC9 (encoding Mut2 PRC1), pDC10 (encoding Mut3 PRC1) and pDC13 (encoding wt PRC1) are transformed in the strain W3116KAR2p-lacz (see above) *via* electroporation (see Example 3).

The β-galactosidase expression induced by the mutant PRC1 genes is compared to the strain which harbors the wt gene. Three individual transformants from each of the above four transformations are grown as in Example 4. Each assay is performed in duplicate. The results are depicted in Table 5. The β-galactosidase activity in the strain transformed with pDC13, which encodes wt PRC1, is set to the value 1.

TABLE5. Induction of β-galactosidase by mutated pro-CPY molecules in a strain which harbors KAR2p-lacz

Plasmid	PRC1 allele	β-gal activity
pDC13	wt	1
pDC8	Mut1	1.5
pDC9	Mut2	2
pDC10	Mut3	1.2

Example 10: Construction of an expression cassette for the human β-amyloid peptide (βA4), without any signal peptide

The plasmid pJC21 (encoding only βA4 with no signal sequence; expression being under the control of the GAPCL promoter) is constructed as following.

A SalI-BglII fragment of the GAPCL promoter (containing a ~275 bp SalI-BamHI pBR322 fragment and a ~400 bp promoter fragment from the yeast GAPDH gene) is isolated by PCR using the primers with SEQ ID NO 8 and 9. Plasmid pBC1 that is constructed as described in Chaudhuri *et al.*, Eur. J. Biochem. (1992), **206**, 793-800 is used as template.

The double-stranded DNA encoding the 42 amino acid βA4 peptide (Müller-Hill & Beyreuther, Annu. Rev. Biochem. (1989), **58**, 287-307) is chemically synthesized using oligomers with SEQ ID NO 10/11 and is amplified by PCR using two primers (SEQ ID NO 12 and 13). The BglII-EcoRI fragment of βA4 is subcloned in pUC19Bgl (the BamHI site of pUC19 has been modified to a BglII site to form pUC19Bgl; the BamHI site is flushed with Klenow polymerase; BglII linkers /Boehringer are ligated, which is followed by digestion with BglII and religation) and the sequence is confirmed as in Example 2.

SEQ ID NO 10/11

```
    BglII  Met
5'  AGATCTGATG GACGCTGAAT TTAGACACGA CTCTGGTTAC GAAGTTCACC

    ACCAAAAGTT GGTCTTCTTC GCTGAAGACG TTGGT                    3' (SEQ ID 10)
3'             CCAGAAGAAG CGACTTCTGC AACCAAGATT GTTCCCACGA

    TAATAACCAA ACTACCAACC ACCACAACAC TAGCGAATTC TTAAG          5' (SEQ ID 11)
                                              EcoRI
```

An EcoRI-SacI terminator fragment from the yeast SUC2 gene (Taussig & Carlson, Nucleic Acids Res. (1983), **11**, 1943-1954) is isolated by PCR using the primers with SEQ ID NO 14 and 15. Yeast genomic DNA (from the wild type strain S288C) is used as template.

The three fragments (SalI-BglII, BglII-EcoRI and EcoRI-SacI) are subcloned in pDP34, which is completely digested with SalI and SacI, to yield pJC21 (see Table 6).

Example 11: Construction of expression cassettes for the human β-amyloid peptide (βA4), with a signal peptide

pJC22 (encoding the SUC2 signal sequence linked to βA4; expression being under the control of the GAPCL promoter), pJC26 (encoding the PRC1 prepro-sequence linked to βA4; expression being under the control of the PRC1 promoter) and pDP34-NLS-βA4

(encoding the nuclear localization sequence, NLS, from the SV40 large T antigen sequence and βA4; expression being under the control of the GAPCL promoter) are constructed in a way similar to the construction of pJC21 (see Example 10; Table 6).

A SalI-BglII fragment containing the GAPCL promoter linked to the SUC2 signal sequence (i. e. pBR322-GAPCLp-Invss) is amplified by PCR using the primers with SEQ ID NO 8 and 16. Plasmid pBC1 (see Example 10) is used as template.

A SalI-BglII fragment containing the PRC1 promoter linked to the prepro sequence of CPY (i. e. CPYp-prepro) is amplified by PCR using the primers with SEQ ID NO 17 and 18, the template being pLV9 (see Example 5 and 6).

A SalI-BglII fragment (i. e. pBR322-GAPCLp-NLS) containing the GAPCL promoter linked to the nuclear localization sequence from SV40 T antigen is amplified by PCR using the primers with SEQ ID NO 8 and 19. The template for the PCR is the plasmid pRH3 which encodes the ~275 bp SalI-BamHI fragment from pBR322, a ~400 bp BamHI-EcoRI GAPCLp fragment and an EcoRI-SpeI fragment of the nuclear localization sequence from SV40 T antigen. This nuclear localization sequence (Nelson & Silver, Mol. Cell. Biol. (1989) 9, 384-389) has been chemically synthesized using the deoxyoligoribonucleotide with SEQ ID NO 30.

SEQ ID NO 30

```
   EcoRI M   D   K   V   F   R   N   S   S   R   T   P   P
5' AATTC ATG GAC AAG GTC TTC AGA AAC TCT TCC AGA ACT CCA CCA
3'     G TAC CTG TTC CAG AAG TCT TTG AGA AGG TCT TGA GGT GGT

K  K   K   R   K   V   E   D   P   A
AAG AAG AAG AGA AAG GTT GAA GAC CCA GCA                    3'
TTC TTC TTC TCT TTC CAA CTT CTG GGT CGT GATC                5'
                                  SpeI
```

One of the above SalI-BglII fragments, the BglII-EcoRI fragment (encoding βA4, see Example 10) and EcoRI-SacI fragment (encoding the SUC2 terminator, see Example 10) are subcloned in pDP34 (completely digested with SalI and SacI) to yield pJC22, pJC26 and pDP34-NLS-βA4, respectively.

Table 6. βA4 expression cassettes of βA4 with and without signal peptides

Plasmid Name	Expression Cassette	Assembled from the Fragments
pJC21	GAPCLp- βA4-SUC2t	SalI-BglII GAPCLp + BglII-EcoRI βA4 (chemically synthesized) + EcoRI-SacI
pJC22	GAPCLp-invss-βA4-SUC2t	SalI-BglII GAPCLp-invss+ BglII-EcoRI βA4 (chemically synthesized) + EcoRI-SacI SUC2t
pJC26	CPYp-preproCPY-βA4-SUC2t	SalI-BglII CPYp-preproCPY+ BglII-EcoRI βA4 (chemically synthesized) + EcoRI-SacI SUC2t
pDP34-NLS- βA4	GAPCLp-NLS βA4-SUC2t	SalI-BglII GAPCLp-invss+ BglII-EcoRI βA4 (chemically synthesized) + EcoRI-SacI SUC2t

Example 12: Yeast transformation of pJC21, pJC22, pJC26 and pDP34-NLS- βA4 and β-galactosidase assay of transformants

The plasmids are transformed in the strain W3116-3 × lacz (see Example 3). β-galactosidase activity is measured (as in Example 4), see Table 7.

Table 7. Only βA4 in the secretory pathway registers as malfolded in the UPR-lacz assay

Plasmid Name	Expression Cassette	W3116-3 × lacz Transformant	β-gal activity
pJC21	GAPCLp-βA4-SUC2t	YJC21	1
pJC22	GAPCLp-invss-βA4-SUC2t	YJC22	3
pJC26	CPYp-preproCPY-βA4-SUC2t	YJC26	4
pDP34-NLS-βA4	GAPCLp-NLS βA4-SUC2t	3 × lacz-NLS-βA4	1

Example 13: Construction of C-terminal truncations of βA4; yeast transformation; β-galactosidase assay

Two plasmids pJC24 (encoding the SUC2 signal sequence and a truncated version of βA4, 1-39) and pJC25 (encoding the SUC2 signal sequence and a truncated version of βA4, 1-

36) are constructed as described below. Both expression cassettes are under the control of the GAPCL promoter. The peptides βA4, 1-39 and βA4, 1-36 have slower rates of aggregation in vitro (Burdick *et al.*, J. Biol. Chem. (1992), **267**, 546-554; Barrow *et al.*, Mol. Biol. (1992), **225**, 1075-1093; C. Pike *et al.*, J. Neuroscience (1993), **13**, 1676-1687).

Two SalI-XbaI fragments, pBR322-GAPCLp-Invss-βA4 1-39 and pBR322-GAPCLp-Invss-βA4,1-36 are isolated by PCR using the two primers (SEQ ID NO 8 and 20 for the former and SEQ ID NOs 8 and 21 for the latter construct). The template is pJC22.

An XbaI-SacI terminator fragment from the yeast SUC2 gene is amplified by PCR using yeast genomic DNA (from the wild type strain S288C) as template. The primers have SEQ ID NOs 22 and 15.

The SalI-XbaI fragment and an XbaI-SacI fragment is subcloned in pDP34 completely digested with SalI and SacI (see Table 8).

Table 8. Expression cassettes for C-terminal truncated versions of βA4

Plasmid Name	Expression Cassette	Assembled from the Fragments
pJC24	GAPCLp-invss-βA4(1-39)-SUC2t	SalI-XbaI GAPCLp-invss-βA4 1-39 (PCR product) + XbaI-SacI SUC2t
pJC25	GAPCLp-invss-βA4(1-36)-SUC2t	SalI-XbaI GAPCLp-invss-βA4 1-36 (PCR product) + XbaI-SacI SUC2t

The plasmids are transformed in the strain W3116-3 × lacz (see Example 3). β-galactosidase activity is measured (as in Example 4) and compared with the strains YJC21 and YJC22, described in Example 10 (see Table 9).

Table 9. C-terminal truncated βA4 molecules are less malfolded than wt βA4 in the UPR-lacz assay

Plasmid Name	Expression Cassette	W3116-3 × lacz Transformant	β-gal activity
pJC24	GAPCLp-invss-βA4(1-39)-SUC2t	YJC24	1.8
pJC25	GAPCLp-invss-βA4(1-36)-SUC2t	YJC25	2.2
pJC21	GAPCLp-βA4-SUC2t	YJC21	1
pJC22	GAPCLp-invss-βA4-SUC2t	YJC22	3

Example 14: Construction of the rat mutant βA4 (mr βA4) and the inverted human βA4 (iβA4)

The plasmids pJC27 and pJC28 are constructed as shown in Table 10. Both expression cassettes contain the prepro sequence of the PRC1 gene and are driven by the PRC1 promoter. pJC27 encodes the rat mutant βA4 (mrβA4) (Dyrks *et al.*, FEBS Lett. (1993), **324**, 231-236; Hilbich *et al.*, Mol. Biol. (1991), **218**, 149-163) and pJC28 encodes the inverted human βA4 (iβA4) (Fraser *et al.*, Biochemistry (1992), **31**, 10716-10723).

As described in Table 10, the plasmids are constructed of a SalI-BglII fragment (containing the CPY promoter and the prepro sequence of CPY), a BglII-EcoRI fragment (containing either mrβA4 or iβA4) and an EcoRI-SacI fragment of the SUC2 terminator.

The BglII-EcoRI fragments (containing either mrβA4 or iβA4) are synthesized using two overlapping oligodeoxyribonucleotides (see SEQ ID NOs 25/26 and 27/28) and two primers (see SEQ ID NOs 12 and 13), which hybridize to the 5' and 3' end of the fragments.

The SalI-BglII, the BglII-EcoRI and the EcoRI-SacI fragment is subcloned in pDP34 completely digested with SalI and SacI.

Table 10. βA4 expression cassettes for the rat mutant βA4 (mrβA4) and the inverted human βA4 (iβA4)

Plasmid Name	Expression Cassette	Assembled from the Fragments
pJC27	CPYp-preproCPY-mrβA4-SUC2t	SalI-BglII CPYp-preproCPY (Example 11) + BglII-EcoRI mr βA4 (synthesized by PCR using the oligomers with SEQ ID NO 23/24, 12,13) + EcoRI-SacI SUC2t (Example 10)
pJC28	CPYp-preproCPY-iβA4-SUC2t	SalI-BglII CPYp-preproCPY (Example 11) + BglII-EcoRI iβA4 (synthesized; by PCR using the oligomers with SEQ ID NO 25/26, 12, 13) + EcoRI-SacI SUC2t (Example 10)

The amino acid sequences of βA4, mrβA4 and iβA4 are shown in Table 11. The differences from the wt human βA4 sequence are underlined.

SEQ ID NOs 23/24

```
    BglII  Met
5'  AGATCTGATG GACGCTGAAT TTGGTCACGA CTCTGGTTTC GAAGTTAGAC

    ACCAAAAGTT GGTTATCGGT GCTGAAGACG TTGGT                    3' (SEQ ID 23)
3'             CCAATAGCCA CGACTTCTGC AACCAAGGTT GTTCCCACGA

    TAGTAACCAA ACTACCAACC ACCACAACAG TAGCGAACTC TTAAG         5' (SEQ ID 24)
                                                 EcoRI
```

SEQ ID NOs 25/26

```
    BglII  Met
5'  AGATCTGATG GCTATCGTTG TCGGTGGTGT TATGTTGGGT ATCATTGCTG

    GTAAGAACTC TGGTGTTGAC GAAGCTTTCT TCGTT                    3' (SEQ ID 25)
3'             ACCACAACTG CTTCGAAAGA AGCAAAACTT CGTTGTGGTG

    CAACTTATGC CAAGACTGGT GTCTAAGCTT CGACTGACTC TTAAG         5' (SEQ ID 26)
                                                 EcoRI
```

Table 11. The βA4, mrβA4 and iβA4 peptide sequences (SEQ ID NOs 27, 28 and 29)

βA4	DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA	SEQ ID 27
mrβA4	DAEFGHDSGF EVRHQKLVIG AEDVGSNKGA IIGLMVGGVV IA	SEQ ID 28
iβA4	AIVVGGVMLG IIAGKNSGVD EAFFVLKQHH VEYGSDHRFE AD	SEQ ID 29

The plasmids in Table 10 are transformed in the strain W3116-3 × lacz (see Example 3). β-galactosidase activity is measured as in Example 4 and compared with the strains YJC26, YJC21 and YJC22, described in Example 10 (see Table 12).

Table 12. The rat mutant βA4 (mr βA4) and the inverted human βA4 (iβA4) βA4 molecules are not malfolded in the UPR-lacz assay

Plasmid Name	Expression Cassette	W3116-3 × lacz Transformant	β-gal activity
pJC27	CPYp-preproCPY-mrβA4-SUC2t	YJC27	1.2
pJC28	CPYp-preproCPY-iβA4-SUC2t	YJC28	1
pJC26	CPYp-preproCPY-βA4-SUC2t	YJC26	4
pJC22	GAPCLp-invss-βA4-SUC2t	YJC22	3
pJC21	GAPCLp-βA4-SUC2t	YJC21	1

Example 15: Trivalent aluminum and divalent zinc increase malfolding of βA4 in yeast

The cations Al^{3+} and Zn^{2+} are known to induce aggregation of βA4 in vitro (Mantyh *et al.*, J. Neurochemistry (1993), 61, 1171-1174). β-galactosidase activity is measured as in Example 4 after cells are grown in YPD in the presence of $AlCl_3$ (~3 mM; Merck) and $ZnCl_2$ (~300μM; Merck). The results are described in Table 13. β-GA represents the β-galactosidase activity. The symbol "-ive control" implies that the cells have been grown in the absence of either of the two transition metal cations.

Table 13. Al^{3+} and Zn^{2+} induce more malfolding in βA4 than in the βA4 variants

Plasmid Name	Expression Cassette	W3116-3× lacz Transformant	β-GA -ive Control	β-GA +$AlCl_3$	β-GA +$ZnCl_2$
pJC26	CPYp-preproCPY-βA4-SUC2t	YJC26	4	9	5.5
pJC22	GAPCLp-invss-βA4-SUC2t	YJC22	3	7	4
pJC21	GAPCLp-βA4-SUC2t	YJC21	1	1.6	1.1
pJC24	GAPCLp-invss-βA4(1-39)-SUC2t	YJC24	1.8	2	1.5
pJC25	GAPCLp-invss-βA4(1-36)-SUC2t	YJC25	2.2	3.5	2.7
pJC27	CPYp-preproCPY-mrβA4-SUC2t	YJC27	1.2	1.5	n.d.
pJC28	CPYp-preproCPY-iβA4-SUC2t	YJC28	1	1.3	n.d.

Example 16: Desferal and ascorbic acid reduce malfolding of βA4

βA4 is expressed in the minimal medium SD (see Example 4) in the absence or presence of desferrioxamine (Desferal® CIBA-GEIGY) and ascorbic acid (Merck). β-galactosidase activity is measured as in Example 4. The results are described in Tables 14 and 15. β-GA represents the β-galactosidase activity. The symbol "-ive control" implies that the cells have been grown in the absence of either desferrioxamine or ascorbic acid.

Table 14. Desferal prevents malfolding of βA4

Plasmid Name	Expression Cassette	W3116-3× lacz Transformant	β-GA -ive Control	β-GA + Desferal 4.5μM	β-GA + Desferal 4.5μM + Fe^{3+} 5μM
pJC26	CPYp-preproCPY-βA4-SUC2t	YJC26	4	1.4	3.5
pJC21	GAPCLp-βA4-SUC2t	YJC21	1	1	1

Table 15. Ascorbic acid also prevents malfolding of βA4

Plasmid Name	Expression Cassette	W3116-3×lacz Transformant	β-GA -ive Control	β-GA + Ascorbic Acid 1mM
pJC26	CPYp-preproCPY-βA4-SUC2t	YJC26	4	1.9
pJC21	GAPCLp-βA4-SUC2t	YJC21	1	1

It appears that both DFO and ascorbic acid reduce malfolding/ aggregation of βA4 in the yeast cell.

Depositions

The following microorganism strains were deposited at the Deutsche Sammlung von Mikroorganismen (DSM), Mascheroder Weg 1b, D-38124 Braunschweig (accession numbers and deposition dates given):

E. coli JM109/pDP34 DSM4473 March 14, 1988

Sequence Listing

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: CIBA-GEIGY AG
(B) STREET: Klybeckstr. 141
(C) CITY: Basel
(E) COUNTRY: Switzerland
(F) POSTAL CODE (ZIP): 4002
(G) TELEPHONE: +41 61 69 11 11
(H) TELEFAX: + 41 61 696 79 76
(I) TELEX: 962 991

(ii) TITLE OF INVENTION: Method for the detection of malfolded proteins

(iii) NUMBER OF SEQUENCES: 30

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 94810536.6
(B) FILING DATE: 16-SEP-1994

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION: 5..27
(D) OTHER INFORMATION: /product= "UPR element"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..4
(D) OTHER INFORMATION: /function= "XhoI linker"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 28..31
(D) OTHER INFORMATION: /function= "XhoI linker"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TCGAGGGAAC TGGACAGCGT GTCGAAATCG A 31

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..45
(D) OTHER INFORMATION: /function= "primer for site directed mutagenesis"
/note= "sense strand of the yeast PRC1 gene for PCR reaction"

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(B) LOCATION: 4..9
(D) OTHER INFORMATION: /function= "MunI site"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 21..23
(D) OTHER INFORMATION: /function= "mutation in respect to wild type"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ACGCAATTGA AAACTATCAG AAGCGTGTCA ACAACAAGAT TAAGG 45

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: complement (1..27)
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "anti-sense strand of the yeast PCR1 gene
for PCR reaction"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 5..10
(D) OTHER INFORMATION: /function= "BamHI site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GACCGGATCC TTTGCAGGAT CGTTTCT 27

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..41
(D) OTHER INFORMATION: /function= "primer for site directed mutagenesis"
/note= "sense strand of yeast PCR1 gene for PCR reaction"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 4..9
(D) OTHER INFORMATION: /function= "MunI site"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: group(16..19, 21..24)
(D) OTHER INFORMATION: /function= "mutations in respect to wild type"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ACGCAATTGA AAACTTGGAC AAGCGTGTCA ACAACAAGAT T 41

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..47
(D) OTHER INFORMATION: /function= "primer for site directed mutagenesis"
/note= "sense strand of the yeast PCR1 gene for PCR reaction"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 4..9
(D) OTHER INFORMATION: /function= "MunI site"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: group(18, 22, 24..32)
(D) OTHER INFORMATION: /function= "mutations in respect to wild type"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ACGCAATTGA AAACTATAAG CGTGACCCAG GTAAGATTAA GGACCCT 47

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..28

(D) OTHER INFORMATION: /function= "PCR primer"
/note= "sense strand of the promoter sequence of the yeast KAR2 gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3..8
(D) OTHER INFORMATION: /function= "SalI site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

TAGTCGACAC TTCAATGTCT AATGCTAG 28

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: complement (1..25)
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "anti-sense strand of the promoter sequence of the yeast KAR2 gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3..8
(D) OTHER INFORMATION: /function= "BamHI site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TAGGATCCGG TATGTTTGAT ACGCT 25

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..28
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "sense strand of the pBR322 sequence"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3..8
(D) OTHER INFORMATION: /function= "SalI site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TAGTCGACGC TCTCCCTTAT GCGACTCC 28

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(B) LOCATION: complement (1..28)
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "anti-sense strand of the promoter sequence
of the yeast GAPDH gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3..8
(D) OTHER INFORMATION: /function= "BglII site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ATAGATCTTT TGTTTATGTG TGTTTATT 28

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..85
(D) OTHER INFORMATION: /function= "DNA with sticky ends"
/note= "sense strand of the human betaA4 peptide"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..6
(D) OTHER INFORMATION: /function= "BglII site"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 61..85

(D) OTHER INFORMATION: /function= "overlap with SEQ ID NO 11"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

AGATCTGATG GACGCTGAAT TTAGACACGA CTCTGGTTAC GAAGTTCACC ACCAAAAGTT 60

GGTCTTCTTC GCTGAAGACG TTGGT 85

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: complement (1..85)
(D) OTHER INFORMATION: /function= "second part of DNA with sticky ends"
/note= "anti-sense strand of human betaA4"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 79..85
(D) OTHER INFORMATION: /function= "EcoRI site"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..25
(D) OTHER INFORMATION: /function= "overlap with SEQ ID NO 10"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CCAGAAGAAG CGACTTCTGC AACCAAGATT GTTCCCACGA TAATAACCAA ACTACCAACC 60

ACCACAACAC TAGCGAATTC TTAAG 85

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..19
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "sense strand of human betaA4"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3..8
(D) OTHER INFORMATION: /function= "BglII site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

ATAGATCTGA TGGACGCTG 19

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: complement (1..20)
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "anti-sense strand of human betaA4"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3..8
(D) OTHER INFORMATION: /function= "EcoRI site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

ATGAATTCTC AAGCGATGAC 20

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..28
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "sense strand of yeast SUC2 gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3..8
(D) OTHER INFORMATION: /function= "EcoRI site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

ATGAATTCAG GTTATAAAAC TTATTGTC 28

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: complement (1..26)
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "anti-sense strand of yeast SUC2 gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3..8
(D) OTHER INFORMATION: /function= "SacI site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TAGAGCTCGG TCCATCCTAG TAGTGT 26

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(B) LOCATION: complement (1..30)
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "anti-sense strand of yeast SUC2 signal sequence"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3..8
(D) OTHER INFORMATION: /function= "BglII site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

ATAGATCTGC TGCAGATATT TTGGCTGCAA 30

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..28
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "sense strand of the promoter sequence of the yeast PRC1 gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3..8
(D) OTHER INFORMATION: /function= "SalI site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

ATGTCGACAT CGATTTCCGT ATATGATG 28

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: complement (1..30)
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "anti-sense strand of the yeast PRC1 gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3..8
(D) OTHER INFORMATION: /function= "BglII site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

ATAGATCTTT AATCTTGTTG ACACGAAGCT 30

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(B) LOCATION: complement (1..29)
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "anti-sense strand of the SV40 T antigen nuclear localization signal"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3..8
(D) OTHER INFORMATION: /function= "BglII site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

ATAGATCTGC TGGGTCTTCA ACCTTTCTC 29

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: complement (1..29)
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "anti-sense strand of human betaA4"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3..8
(D) OTHER INFORMATION: /function= "XbaI site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

ATTCTAGATT AAACACCACC AACCATCAA 29

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: complement (1..29)
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "anti-sense strand of human betaA4"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3..8
(D) OTHER INFORMATION: /function= "XbaI site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

ATTCTAGATT AAACCATCAA ACCAATAAT 29

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(B) LOCATION: 1..28
(D) OTHER INFORMATION: /function= "PCR primer"
/note= "sense strand of yeast SUC2 gene"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 2..8
(D) OTHER INFORMATION: /function= "XbaI site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

ATTCTAGAAG GTTATAAAAC TTATTGTC 28

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..85
(D) OTHER INFORMATION: /function= "part of DNA with sticky ends"
/note= "sense strand of mutant rat betaA4"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..6
(D) OTHER INFORMATION: /function= "BglII site"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 61..85

(D) OTHER INFORMATION: /function= "overlap with SEQ ID NO 24"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

AGATCTGATG GACGCTGAAT TTGGTCACGA CTCTGGTTTC GAAGTTAGAC ACCAAAAGTT 60

GGTTATCGGT GCTGAAGACG TTGGT 85

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: complement (1..85)
(D) OTHER INFORMATION: /function= "second part of DNA with sticy ends"
/note= "overlaps with SEQ ID NO 23 of mutant rat betaA4"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 79..85
(D) OTHER INFORMATION: /function= "EcoRI site"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..25
(D) OTHER INFORMATION: /function= "overlap with SEQ ID NO 23"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CCAATAGCCA CGACTTCTGC AACCAAGGTT GTTCCCACGA TAGTAACCAA ACTACCAACC 60

ACCACAACAG TAGCGAACTC TTAAG 85

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..85
(D) OTHER INFORMATION: /function= "DNA with sticky ends"
/note= "sense strand of inverted human betaA4
using yest codons"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..6
(D) OTHER INFORMATION: /function= "BglII site"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 61..85
(D) OTHER INFORMATION: /function= "overlap with SEQ ID NO 26"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

AGATCTGATG GCTATCGTTG TCGGTGGTGT TATGTTGGGT ATCATTGCTG GTAAGAACTC 60

TGGTGTTGAC GAAGCTTTCT TCGTT 85

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: complement (1..85)
(D) OTHER INFORMATION: /function= "second part of DNA with sticky ends"
/label= ibetaA4
/note= "overlaps with SEQ ID NO 25, inverted human betaA4"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..25
(D) OTHER INFORMATION: /function= "overlap with SEQ ID NO 25"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 79..85
(D) OTHER INFORMATION: /function= "EcoRI site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

ACCACAACTG CTTCGAAAGA AGCAAAACTT CGTTGTGGTG CAACTTATGC CAAGACTGGT 60

GTCTAAGCTT CGACTGACTC TTAAG 85

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..42
(D) OTHER INFORMATION: /label= betaA4
/note= "human betaA4"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30

Gly Leu Met Val Gly Gly Val Val Ile Ala
35 40

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..42
(D) OTHER INFORMATION: /label= mrbetaA4
/note= "mutant rat betaA4"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Asp Ala Glu Phe Gly His Asp Ser Gly Phe Glu Val Arg His Gln Lys
1 5 10 15

Leu Val Ile Gly Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30

Gly Leu Met Val Gly Gly Val Val Ile Ala
35 40

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..42
(D) OTHER INFORMATION: /label= ibetaA4
/note= "inverted human betaA4"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Ala Ile Val Val Gly Gly Val Met Leu Gly Ile Ile Ala Gly Lys Asn
1 5 10 15

Ser Gly Val Asp Glu Ala Phe Phe Val Leu Lys Gln His His Val Glu
20 25 30

Tyr Gly Ser Asp His Arg Phe Glu Ala Asp
35 40

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..5
(D) OTHER INFORMATION: /function= "EcoRI site overhang"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 6..74
(D) OTHER INFORMATION: /product= "Nuclear localization signal from SV40 T antigen"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 75..78
(D) OTHER INFORMATION: /function= "SpeI site overhang"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

AATTCATGGA CAAGGTCTTC AGAAACTCTT CCAGAACTCC ACCAAAGAAG AAGAGAAAGG 60

TTGAAGACCC AGCACTAG 78

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 26, line 10 - 14.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
DSM Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b
D-38124 Braunschweig

Date of deposit	Accession Number
14 March 1988 (14.03.88)	DSM 4473

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

We request the Expert Solution where available.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☒ This sheet was received with the international application 04.09.95	☐ This sheet was received by the International Bureau on:
Authorized officer O. GORGE	Authorized officer

Form PCT/RO/134 (July 1992)

Claims

1. A host transformed with at least a first and a second expression cassette, wherein
   - the first expression cassette comprises one or more unfolded-protein-response elements (UPR) operably linked to a reporter element;
   - the second expression cassette comprises a promoter operably linked to a signal sequence, to a DNA encoding a protein whose malfolding is to be monitored and to a terminator; and

   wherein these first and second expression cassettes are not naturally occurring in the host and wherein the protein to be monitored in the second expression cassette is selected from the group consisting of a prion, p53, β-amyloid peptide and functional derivatives thereof.

2. A host according to claim 1 which is capable of secreting proteins.

3. A host according to claim 1 which is a plant, insect, mammalian, fungal, or animal cell.

4. A host according to claim 1 which is a fungal cell.

5. A host according to claim 1 which is yeast cell.

6. A host according to claim 1 which is *Saccharomyces cerevisiae*.

7. A host according to claim 1, characterized in that the UPR of the first expression cassette originates from BiP.

8. A host according to claim 1, characterized in that the UPR of the first expression cassette comprises the DNA sequence as depicted in SEQ ID NO 1 or a functional equivalent thereof.

9. A host according to claim 1, characterized in that the UPR is present in more than one copy in the first expression cassette.

10. A host according to claim 1, characterized in that the UPR is present in 2 to 5 copies in the first expression cassette.

11. A host according to claim 1, characterized in that the reporter element comprises a promoter operably linked to a DNA that is transcribed under the control of this promoter, and to a terminator.

12. A host according to claim 11, characterized in that the promoter in the first expression cassette is tightly regulated.

13. A host according to claim 12, characterized in that the promoter is selected from the group consisting of CYC1 and KAR2 promoter.

14. A host according to claim 1, characterized in that the transcription of the reporter element causes an effect that can be measured easily during or after transcription or translation.

15. A host according to claim 1, characterized in that the reporter element codes for a protein whose amount can be determined easily.

16. A host according to claim 15, characterized in that the reporter protein is selected from the group consisting of β-galactosidase and luciferase.

17. A host according to claim 11, characterized in that the terminator is selected from the group consisting of the terminator naturally linked to the transcribed DNA, the terminator of the PHO5, of the α-factor and of the SUC2 gene.

18. A host according to claim 1, characterized in that the promoter of the second expression cassette is an inducible promoter.

19. A host according to claim 1, characterized in that the promoter of the second expression cassette is selected from the group consisting of the promoter originally linked to the DNA encoding the protein whose malfolding is to be monitored, the CUP1, GAPDH, GAPFL, GAL(10), PYK, TPI, ADH, PRC1 and PGK promoter.

20. A host according to claim 1, characterized in that the promoter of the second expression cassette is the GAPFL promoter or a functional derivative thereof.

21. A host according to claim 1, characterized in that the signal sequence of the second expression cassette is selected from the group consisting of the SUC2, the α-factor, KEX1, PHO5 and the glucoamylase signal sequence.

22. A host according to claim 1, characterized in that the signal sequence of the second expression cassette is the SUC2 or the α-factor signal sequence.

23. A host according to claim 1, characterized in that the protein to be monitored in the second expression cassette has an unusual amount of hydrophobic groups at the surface.

24. A host according to claim 1, characterized in that the protein to be monitored in the second expression cassette is or comprises the β-amyloid peptide or a functional derivative thereof.

25. A host according to claim 1, characterized in that the terminator of the second expression cassette is selected from the group consisting of the terminator naturally adjacent to the DNA coding for the protein, the α-factor terminator and the SUC2-terminator.

26. An expression cassette according to claim 1, comprising a promoter operably linked to a signal sequence, a DNA encoding the β-amyloid peptide or a functional derivative thereof, and to a terminator.

27. A hybrid plasmid comprising an expression cassette according to claim 2 6.

28. A hybrid plasmid according to claim 27, characterized in that it is based on the two-micron plasmid of *S. cerevisiae* .

29. A method for the determination of the influence of a compound on the appearance of malfolded protein, comprising culturing a transformed host according to claim 1 under suitable conditions, applying the compound to be tested and measuring the amount of reporter gene activation.

30. A method according to claim 29 comprising the steps

- growing the transformed host according to claim 1 under suitable conditions,

- optionally inducing the expression of protein whose malfolding is to be monitored,
- optionally adding a compound that promotes the malfolding or aggregation,
- adding the test compound, and
- monitoring the transcription or translation of the reporter element, or the transcription or translation products or the effects caused thereby.

31. A method according to claim 29, for the identification of compounds that inhibit the aggregation of β-amyloid peptide.

32. A compound identified using the method according to claim 29.

33. Use of a compound according to claim 32 in a method of treatment.

34. Use of a compound according to claim 32 for the inhibition of protein aggregation.

35. Use of a compound according to claim 32, for the treatment of Alzheimer's disease.

Figure 1

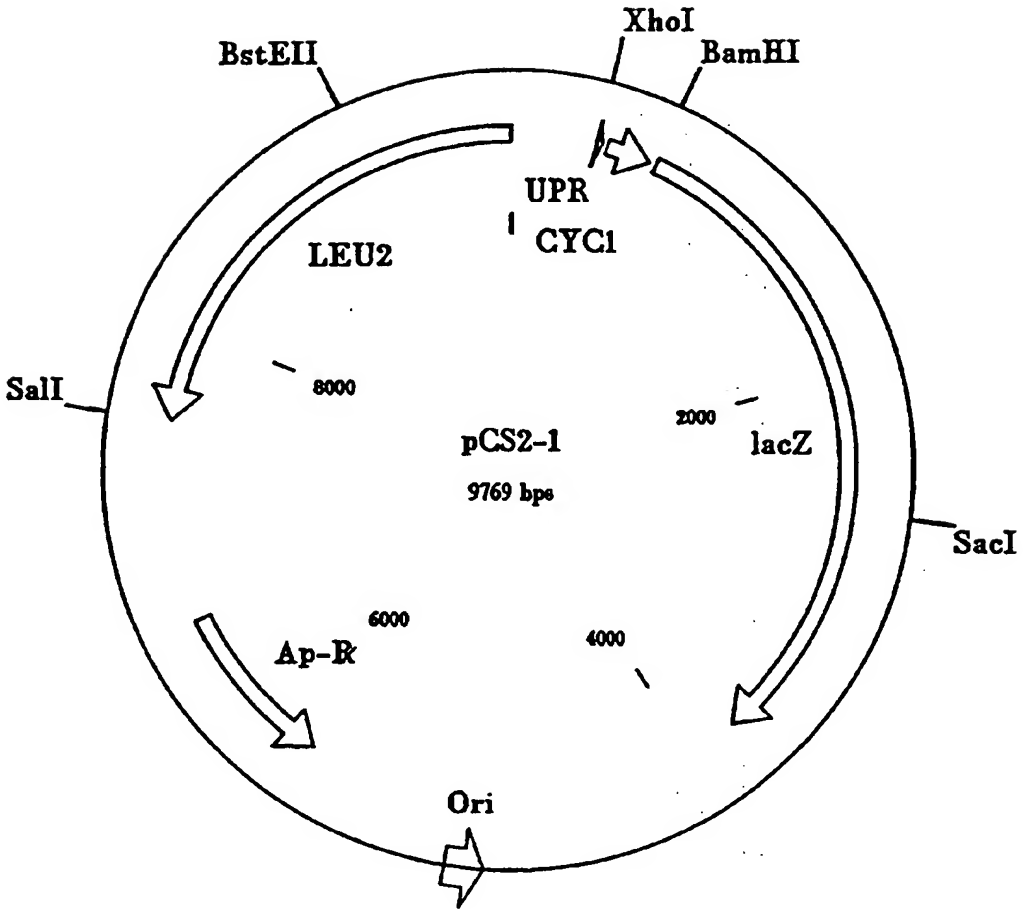
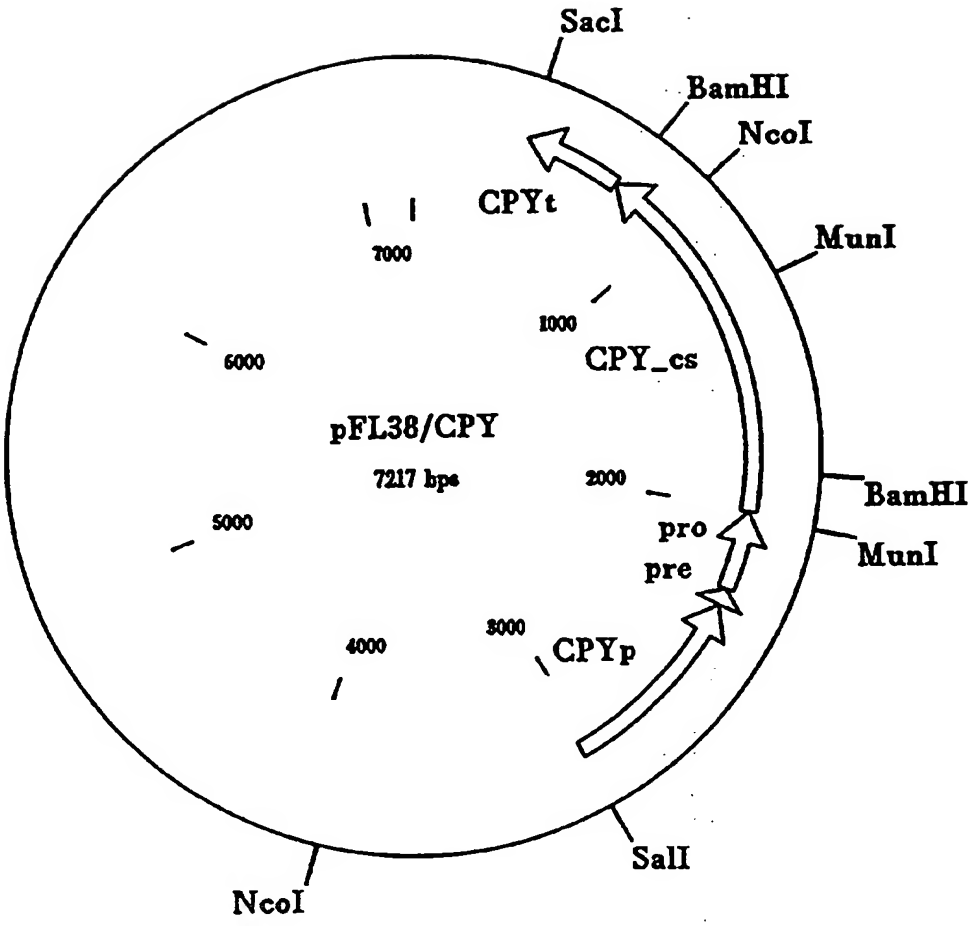

XhoI
BstEII
BamHI
UPR
CYC1
LEU2
SalI
8000
2000
pCS2-1
9769 bps
lacZ
SacI
6000
4000
Ap-R
Ori

Figure 2


SacI
BamHI
NcoI
MunI
CPYt
7000
1000
CPY_cs
6000
pFL38/CPY
7217 bps
2000
BamHI
5000
pro
MunI
pre
3000
4000
CPYp
SalI
NcoI

INTERNATIONAL SEARCH REPORT

Interr ·al Application No
PCT/EP 95/03475

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N15/62 C12N15/67 C12N15/81 C12N1/19
C12Q1/68 //(C12N1/19,C12R1:865)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MOL. CELL. BIOL., vol. 11, no. 11, November 1991 AM. SOC. MICROBIOL., WASHINGTON, D.C. US;, pages 5612-5623, S.K. WOODEN ET AL. 'Transactivation of the grp78 promoter by malfolded proteins, glycosylation block, and calcium ionophore is mediated through a proximal region containing a CCAAT motif which interacts with CTF/NF-I' see page 5615, left column, paragraph 2 - right column, line 1; figures 1-8; tables 1,2 see page 5619, right column, line 31 - page 5621, left column, line 52 --- -/--	1-16,29, 30

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 December 1995	08.01.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Hornig, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CELL, vol. 65, 31 May 1991 CELL PRESS,CAMBRIDGE,MA,US;, pages 765-774, J. MILNER AND E.A. MEDCALF 'Cotranslation of activated mutant p53 with wild type drives the wild-type p53 protein into a mutant conformation' see the whole document ---	1-16,29, 30
Y	SCIENCE, vol. 252, 14 June 1991 AAAS,WASHINGTON,DC,US, pages 1515-1522, S.B. PRUSINER 'Molecular biology of prion disease' see the whole document ---	1-16,29, 30
Y	NATURE, vol. 370, 11 August 1994 MACMILLAN JOURNALS LTD., LONDON,UK, pages 419-420, K. BEYREUTHER AND C.L. MASTERS 'Catching the culprit prion' see the whole document ---	1-16,29, 30
Y	J. NEUROCHEMISTRY, vol. 61, no. 3, 1993 RAVEN PRESS LTD., NEW YORK, US, pages 1171-1174, P.W. MANTYH ET AL. 'Aluminium, iron, and zinc ions promote aggregation of physiological concentrations of beta-amyloid peptide' cited in the application see the whole document ---	1-16,29, 30
Y	MOL. CELL. BIOL., vol. 13, no. 2, February 1993 AM. SOC. MICROBIOL., WASHINGTON, D.C. US;, pages 877-890, K. KOHNO ET AL. 'The promoter region of the yeast KAR2 (BiP) gene contains a regulatory domain that responds to the presence of unfolded proteins in the endoplasmic reticulum' see page 887, right column, paragraph 2 - page 889, left column, paragraph 1 --- -/--	1-16,29, 30

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 95/03475

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PROC. NATL. ACAD SCI., vol. 90, no. 12, 15 June 1993 NATL. ACAD SCI., WASHINGTON, DC, US;, pages 5450-5454, J.A. PARTALEDIS AND V. BERLIN 'The FKB2 gene of Saccharomyces cerevisiae, encoding the immunosuppressant-binding protein FKBP-13, is regulated in response to accumulation of unfolded proteins in the endoplasmic reticulum' see page 5452, right column, paragraph 2 - page 5454, left column, paragraph 2 ---	1-16,29, 30
Y	EMBO JOURNAL, vol. 11, no. 7, July 1992 IRL PRESS LIM., OXFORD, ENGL.;, pages 2583-2593, K. MORI ET AL. 'A 22 bp cis-acting element is necessary and sufficient for the induction of the yeast KAR2 (BiP) gene by unfolded proteins' cited in the application see page 2588, right column, line 27 - page 2592, right column, line 26 ---	1-16,29, 30
Y	CELL, vol. 74, 27 August 1993 CELL PRESS,CAMBRIDGE,MA,US;, pages 743-756, K. MORI ET AL. 'A transmembrane protein with a cdc2+/CDC28-related kinase activity is required for signalling from the ER to the nucleus' see the whole document ---	1-16,29, 30
A	J. BIOL. CHEM., vol. 266, no. 30, 25 October 1991 AM. SOC. MOL. BIOL., INC.,BALTIMORE, US;, pages 20532-20537, N. LENNY AND M. GREEN 'Regulation of endoplasmatic reticulum stress proteins in COS cells transfected with immunglobulin mu heavy chain cDNA' * the whole document * ---	1-35
A	TRENDS IN CELL BIOLOGY, vol. 4, no. 2, February 1994 ELSEVIER SCIENCE, AMSTERDAM, NL;, pages 56-60, C.E. SHAMU ET AL. 'The unfolded-protein-response pathway in yeast' cited in the application * the whole document * ---	1-35

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 95/03475

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NATURE, vol. 332, 31 March 1988 MACMILLAN JOURNALS LTD., LONDON, UK;, pages 462-464, Y. KOZUTSUMI ET AL. 'The presence of malfolded proteins in the endoplasmatic reticulum signals the induction of glucose-regulated proteins' * the whole document * ---	1-35
T	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS 209 (1). 1995. 31-39. ISSN: 0006-291X, 6 April 1995 CHAUDHURI B ET AL 'The unfolded-protein-response element discriminates misfolding induced by different mutant pro-sequences of yeast carboxypeptidase Y.' see the whole document -----	1-35

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